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# BRAF: A Driver of the Serrated Pathway in Colon Cancer

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**In this issue of *Cancer Cell*, Rad and colleagues report findings that underscore the importance of oncogenic *BRAF* mutation coupled with microsatellite instability, *p16Ink4a* inactivation, and *p53* mutation in the serrated pathway of colon cancer development. These findings provide translational insights into potential therapeutic intervention for *BRAF* mutant colon cancers.**

Colorectal cancer (CRC) is a common malignancy in the United States (US) and worldwide. Whereas CRC incidence has been declining steadily over the past decade in the US, mortality remains high and unchanged with over 50,000 deaths annually, which is attributable largely to complications of metastatic disease.

The molecular genetics of hereditary and sporadic CRC have been a paradigm for cancer biology investigation in general over the last 25 years (Fearon, 2011; Rustgi 2007). The majority of CRCs have either inactivating mutations in the *APC* tumor suppressor gene or activating mutations in the β-catenin gene that result in stabilization and nuclear translocation of β-catenin, which in turn cooperates with TCF transcriptional factors to activate a repertoire of genes involved in cell proliferation and growth. Many CRCs involve subsequent mutations in *KRAS*, *TP53*, and *SMAD4*. Comprehensive genomic analyses are expanding the number of genes identified with somatic mutations (Muzni et al., 2012). These classical CRCs often display chromosomal instability (CIN). Another subset of CRCs displays microsatellite instability (MIN). Inactivation of mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) through mutation or hypermethylation results in MIN. Based on the extent of microsatellite instability (MSI), CRCs can be classified as MSI-high, MSI-low, or microsatellite stable (MSS). CRCs with CIN are often MSS.

A less common molecular pathogenesis pathway of sporadic CRC is the serrated pathway, meaning the progression of sessile serrated adenoma (SSA) and traditional serrated adenomas (TSAs) to colorectal cancer, typically (albeit not exclusively) in the proximal or right colon (Leggett and Whitehall, 2010). The term serrated is used due to the "sawtooth" appearance of the crypt epithelium within these polyps. SSAs are highlighted by the presence of *BRAF* point mutation (V600E), leading to enhanced signaling through MEK and ERK, which occurs also with *KRAS* mutation. Yet, classic adenomas are not associated with *BRAF* mutation. Another remarkable feature of SSAs is the CpG island methylator phenotype (CIMP), which fosters the transition of microvesicular hyperplastic polyps to SSAs and eventually to cancer. One key target appears to be methylation of *MLH1*, resulting in MSI-high colon tumors. Yet, the progression of SSAs to cancer is kept in abeyance by $p16^{INK4a}$-mediated senescence as well as intact p53. Overcoming $p16^{INK4a}$ and p53 tumor suppressor activities are likely critical in the ultimate progression to cancer.

Can the serrated pathway be modeled in vivo? In a comprehensive study by Rad et al. (2013) in this issue of *Cancer Cell*, conditional $Braf^{V637E}$ (the murine counterpart to human $BRAF^{V600E}$) knock-in mice were generated and crossed to *Villin-Cre* transgenic mice such that expression of the oncogene would be restricted to small intestinal and colonic epithelia. The resulting double mutant mice developed serrated polyps, which are characterized by hyperproliferation (expansion to mid-upper crypts) but not apoptosis. In an age-dependent fashion, the hyperplasias progressed to dysplasias, and TSAs, but not SSAs, emerged. This might be attributed to the mouse's small intestine's predilection for the polyps, because SSAs in patients tend to be colonic. As such, the authors refer to the small intestinal lesions as murine serrated adenoma (mSA) with either low-grade dysplasia (mSA-LGD) or high-grade dysplasia (mSA-HGD). In ~16% of the mice, dysplasia progressed to invasive carcinomas (5/31). Not surprisingly, but importantly, nearly 40% (13/33) of the $Braf^{V637E}$ mSAs and cancers were MSI-high; by contrast, the mSHs were either MSS or MSI-low.

Given the long latency for cancer formation, *Villin-Cre;*$Braf^{V637E/+}$*;*$p53^{LSL\text{-}R172H/+}$ mice were generated to evaluate the functional consequences of introducing mutant p53. In this context, 56% of the compound mutant mice between 10–20 months developed carcinomas, with the average number of cancers being 5.2 times higher than in *Villin-Cre;*$Braf^{V637E/+}$ mice. These findings underscore the importance of p53 inactivation in late-stage carcinogenesis in the serrated pathway.

As $p16^{Ink4a}$ was induced in mSA-HGD lesions in this study, the authors generated *Villin-Cre;*$Braf^{V637E/+}$ mice with homozygous $p16^{Ink4a}$ mutation to determine if senescence was present concomitantly. These $p16^{Ink4a}$ -deficient compound mutant mice displayed a substantial increase (6.4 times higher) in carcinomas compared to mice with $p16^{Ink4a}$ expression. Of note, mSAs and carcinomas had elevated ERK activation as well as enhanced WNT pathway activation, as revealed by diffuse or focal nuclear β-catenin accumulation.

Mutations were found in *Apc*, *Ctnnb1*, and *Lrp1b*, all components of WNT signaling, accounting for β-catenin redistribution. In summary, this study establishes genetic proof that *Braf* oncogenic mutation can induce formation of mSHs, mSAs, and a low penetrance of carcinomas, with the latter two lesions displaying MSI-high. The carcinoma progression can be accelerated by *p53* mutation or $p16^{Ink4a}$ inactivation.

How do these findings compare to oncogenic *Kras* mutation in the small intestine and colon? Oncogenic $Kras^{G12D}$ expression driven by a transgenic *Villin* promoter induces a spectrum of lesions, ranging from aberrant crypt foci to invasive adenocarcinomas without acquisition of *Apc* mutations, although there is evidence of occasional *p53* mutation in this context (Janssen et al., 2002). In addition, *CDX2P-G22Cre;*$Kras^{LSL\text{-}G12D}$ mutant mice revealed epithelial hyperplasia and crypt architecture changes in the colon, reminiscent of those seen in human hyperplastic polyps (Feng et al., 2011). However, neither TSAs nor SSAs emerged. More recently, Greten and colleagues found in a thorough study that intestinal epithelia-specific expression of oncogenic $Kras^{G12D}$ in mice induced serrated hyperplasia, which was characterized by $p16^{Ink4a}$ overexpression and senescence induction (Bennecke et al., 2010). Deletion of *Cdkn2a* (the locus encoding $p16^{Ink4a}$ and $p19^{Arf}$) in $Kras^{G12D}$-expressing mice prevented senescence and led to invasive, metastasizing carcinomas. However, unlike the oncogenic BRAF-driven tumors in the Rad et al. (2013) study, these tumors were neither MSI-high nor showed WNT pathway activation, generally speaking. Perhaps, we can surmise two pathways that cause the induction of serrated adenomas, recognizing that geographic location (small intestine versus colon) and meticulous morphological analysis are important to distinguish murine lesions from human lesions. The predominant initiating event is *Braf* mutation; conceivably, intestinal crypts are permissive for this oncogenic insult. *Kras* mutation can occur as an "alternative" pathway, but is, perhaps, less tolerated. Regardless, $p16^{Ink4a}$ induction and likely senescence occur in both settings; overcoming this is critical for carcinoma initiation. However, MSI may be divergent, with MSI-high being much more prevalent in oncogenic BRAF-induced lesions compared to oncogenic Kras-induced lesions. Additionally, amplified MAPK signaling is apparent in both scenarios, which may have an impact on consideration of the types of therapeutic intervention(s).

Rad et al. (2013) demonstrate efficacy with MEK inhibition and combinatorial BRAF/PI3K inhibition in cancer cell lines and xenografts, which are resistant to BRAF inhibitor therapy. This may prove promising given the elusiveness of clinical efficacy with BRAF inhibition in selected colon cancers, to date, which is in contrast to the 50%–60% response of metastatic melanomas (Sullivan and Flaherty, 2013). Indeed, there is intense investigation to understand why resistance to BRAF inhibition is present in colon cancer. One answer may be due to feedback signals resulting in amplified EGFR signaling (Prahallad et al., 2012). Another reason may be due to the persistence of amplified ERK signaling, which may represent a rationale for the ongoing development of ERK-specific inhibitors apart from the implementation of MEK inhibitors. Comparison of the commonalities and differences in BRAF inhibition between CRC and melanoma may help in elucidating mechanisms underlying intrinsic and acquired resistance. Mouse models, like the serrated intestinal cancer model reported by Rad et al. (2013), will surely aid these investigations and support application of that knowledge preclinically and clinically.

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